



Effect of preharvest spraying *Cryptococcus laurentii* on postharvest decay and quality of strawberry



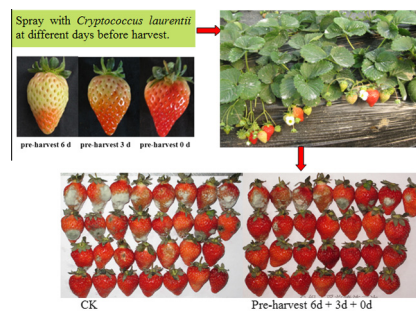
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HIGHLIGHTS

- Preharvest spraying with *C. laurentii* reduced postharvest decay of strawberry fruit.
- The frequency of spraying *C. laurentii* significantly influenced control efficiency.
- Three applications of *C. laurentii* before harvest improved biocontrol efficiency.
- Preharvest treatment with *C. laurentii* did not significantly affect fruit quality.

GRAPHICAL ABSTRACT



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ABSTRACT

Application of a suspension (log 8.0/ml) of *Cryptococcus laurentii* prior to harvest led to a reduction in *Botrytis cinerea* decay of strawberries stored at 4 or 20 °C, for 12 or 4 days, respectively. The frequency of spraying antagonist significantly influenced disease incidence in strawberry fruit. The best inhibition of disease was achieved when fruit sprayed *C. laurentii* with three applications that began 6 days prior to harvest, and the incidence of gray mold and natural decay treated with this method was 21% and 11%, compared with 88% and 62% in the control after storage at 20 °C for 4 days. A similar result occurred in the treated fruit after storage at 4 °C for 12 days. Dilution plate counts on Rose Bengal agar and scanning electron microscopy results showed that three applications with *C. laurentii* at 6, 3 and 0 days before harvest improved its ability to colonize the epidermis of strawberry fruit in the greenhouse and during storage compared to single application. In addition, antagonistic yeast spraying before harvest could reduce the weight loss, delay the decrease of the firmness and ascorbic acid, but had no significant effect on the contents of soluble solids, titratable acidity and fruit surface color. These results suggested that preharvest spraying with *C. laurentii* might be a promising alternative to fungicide application for decay control of strawberry.

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1. Introduction

Strawberry (*Fragaria × ananassa* Duch.) is an important fruit produced in commercial scale in China. Strawberries are highly perishable fruit with a short postharvest life, due to ease of

mechanical injury, physiological disorders, water loss and fungal decay during storage (Garcia et al., 1998). The use of synthetic fungicides such as benzimidazole and dicarboximide is the primary means for reducing postharvest diseases. However, the development of resistance of fungal pathogens to fungicides and public concern over the potential impact of fungicides on human health and environment (Wisniewski and Wilson, 1992; Spadaro et al., 2004; Droby et al., 2009), have created interest in searching for some alternatives to synthetic fungicides.

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Among the proposed non-fungicide methods, biological control using microbial antagonists has shown great potential for control of postharvest disease of fruit and vegetables and is to be regarded as one of the most promising alternatives to reduce synthetic fungicide inputs (Fan and Tian, 2001; Janisiewicz and Korsten, 2002; Zhao et al., 2008; Sharma et al., 2009). Yeasts have been pursued actively in recent years, as production of antibiotics or other toxic secondary metabolites is not involved in their activities against postharvest pathogens (Qin et al., 2006), and considerable information is available with respect to techniques for their genetic manipulation, production and storage (Reeleder, 2004).

In several cases, infection of fruit by postharvest pathogens often occurs in the field (Biggs, 1995), and these latent infections become a major factor for decay during transportation or storage of fruits. Therefore, it would be advantageous to apply antagonists before harvest since they could reduce initial infection and remain active and suppressing pathogens in storage (Teixidó et al., 1998). Preharvest application can enhance the biological system, because it allows the antagonist to have longer interaction with the pathogen and to colonize tissues before the arrival of the pathogen. This is benefited when latent infections and incipient infections occur through wounds during harvesting and packaging period (Ippolito and Nigro, 2000). Field applications of antagonistic microbes are effective to control postharvest decay of fruits and vegetables (Ippolito and Nigro, 2000; Janisiewicz and Korsten, 2002; Zhao et al., 2011). If an antagonist is to have potential for practical use, its ability to colonize the surface of fruit both in the field and storage and to persist for as long as possible is vitally important (Wisniewski and Wilson, 1992). *Cryptococcus laurentii* is well-known biocontrol yeast reported to reduce postharvest disease of fruits (Sharma et al., 2009). It had strong survival ability on fruit surfaces under field conditions, and adaptability to postharvest storage conditions of low temperature, low O₂ and high CO₂ concentrations (Tian et al., 2004). Therefore, it has potential for preharvest application and preharvest application of *C. laurentii* reduced gray mold on pears (Benbow and Sugar, 1999) and *C. laurentii* effectively inhibited postharvest rots of sweet cherry (Tian et al., 2004) and table grapes (Meng et al., 2010).

Although research on preharvest applications of microbial antagonists for controlling fresh fruits postharvest decay are common and the efficacy has been well documented, there is little information about the efficacy of preharvest spraying with *C. laurentii* on postharvest decay and quality of strawberry fruit during storage. Therefore, the objectives of this study were to examine: (1) the effect of preharvest spraying with *C. laurentii* on control of postharvest decay in strawberry fruit during storage; (2) the influence of preharvest spraying with *C. laurentii* on fruit quality; (3) the ability of *C. laurentii* to colonize the surface of strawberry fruit both in the greenhouse and storage.

2. Materials and methods

2.1. Yeast and pathogen

C. laurentii 2.3803 was obtained from the China General Microbiological Culture Collection Center (CGMCC) and was maintained on nutrient yeast dextrose agar (NYDA) medium (containing 8 g nutrient broth, 5 g yeast extract, 10 g glucose and 20 g agar in 1 L of distilled water) at 4 °C before use. Liquid cultures of the yeast were grown in 250 ml Erlenmeyer flasks. Each contained 50 ml of fresh nutrient yeast dextrose broth (NYDB: NYDA without agar), which had been inoculated with a loop of the culture. Flasks were incubated at 28 °C for 24 h on a rotary shaker at 200 rpm. The yeast cells were then harvested by centrifugation at 3000 rpm for 10 min and washed twice with sterile distilled water in order to remove

the residual growth medium. Cell pellets were then re-suspended in sterile distilled water with 0.05% (w/v) Tween-80 and adjusted to the desired concentration of 10⁸ cells ml⁻¹ based on hemocytometer counts of cell density.

Botrytis cinerea was isolated from infected strawberry fruit and preserved on potato dextrose agar (PDA) at 4 °C. Spore suspensions were obtained from 2-week-old cultures. Spore concentrations were adjusted to 1 × 10⁵ spores ml⁻¹ with a hemocytometer.

2.2. Preharvest spray experiment of strawberry

Strawberry (*Fragaria × ananassa* Duch., cultivar 'Toyonoka') was grown according to standard cultural practices in an organic greenhouse located in Nanjing, China. No fungicides were applied prior to harvest.

The preharvest spray experiment was designed according to the method described by Meng et al. (2010). Application of a suspension (log 8.0/ml) of *C. laurentii* prior to harvest was sprayed to run-off on the surface of strawberry fruit by using hand-sprayer. This experiment was divided into four treatments: (A) one application made 6 days prior to harvest; (B) two applications at 6 and 0 days prior to harvest; (C) three applications at 6, 3 and 0 days prior to harvest; and fruit sprayed with sterile distilled water with 0.05% (w/v) Tween-80 were used as the control (CK). The ripeness stage of strawberry during spraying is presented (Fig. 1), according to a previous report (Jia et al., 2011). Every treatment consisted of three replications and each replication contained 30 strawberry plants. The experiment was carried out in duplicate.

Commercial matured fruit were harvested and then transferred immediately to the laboratory. All fruit samples were uniform in size and color, and free of visible physical injury or disease.

2.3. Efficacy of preharvest spraying *C. laurentii* on control of gray mold in strawberry fruit

Strawberry fruit were inoculated by dipping them in the conidial suspension of *B. cinerea* (1 × 10⁵ spores ml⁻¹) for 10 s. All fruit were placed in two layers in plastic boxes wrapped with high density polyethylene sleeve in order to retain a high humidity and stored at 20 °C for 4 days or 4 °C for 12 days. All plastic boxes were put in constant temperature and humidity chambers at RH 90%. The disease incidence of strawberry decay caused by *B. cinerea* was determined after storage. Each treatment contained three replicates with 15 fruit per replicate and the experiment was repeated twice.

2.4. Effect of preharvest spraying *C. laurentii* on natural decay of intact fruit

The effect of preharvest spraying *C. laurentii* on development of natural infection was evaluated according to Zhao et al. (2011), with some modification. Intact fruit were divided into four groups based on preharvest treatments, and then stored at 20 °C for 4 d or 4 °C for 12 d. The percentage of infected fruit was recorded. Each treatment contained three replicates with 15 fruit per replicate and the experiment was repeated twice.

2.5. Effect of preharvest spraying *C. laurentii* on postharvest quality of strawberry fruit

The effect of preharvest spraying *C. laurentii* on postharvest quality of strawberry was determined. Quality parameters were measured on the day of harvest or after storage. There were three replications of 10 fruit each for the assays, and the experiment was repeated twice. All assays were performed at ambient temperature

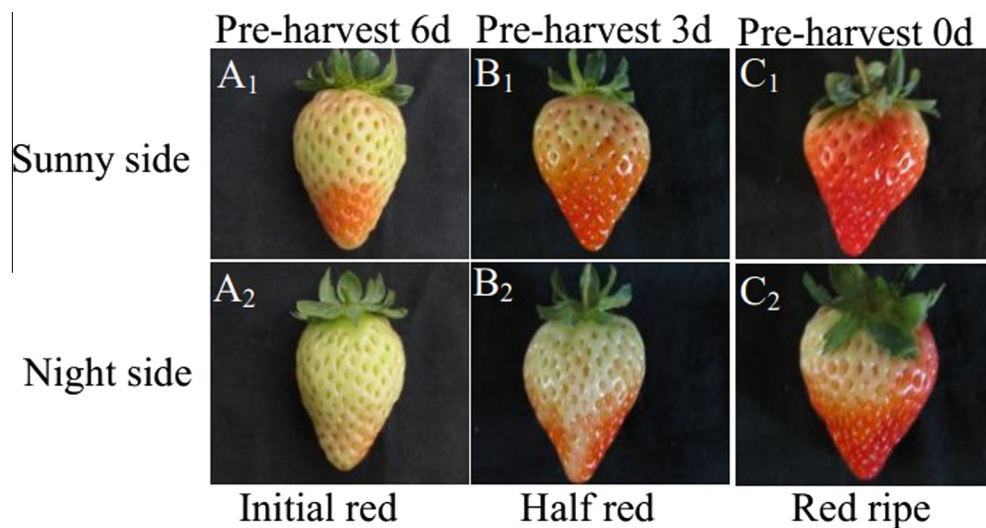


Fig. 1. The maturity stage of strawberry fruit during preharvest spraying. A₁–A₂: at 6 d before harvest, strawberry is in initial red stage that fruit surface begin to turn red; B₁–B₂: at 3 d before harvest, strawberry is in half red stage that half of fruit surface are turned red; C₁–C₂: at commercial harvest day, strawberry fruit is in red ripe stage that three-quarters of fruit surface are turned red.

(approximately 20 °C). The testing methods used are described below.

2.5.1. Fruit firmness

Firmness was determined using Texture Analyzer (TA-XT2; Stable Micro Systems Ltd., UK) with a 5 mm diameter flat probe. Firmness values of each strawberry were measured at two points of the equatorial region. The probe descended towards the sample at 1.0 mm s⁻¹ and the maximum force was recorded as fruit's firmness (N).

2.5.2. Total soluble solids

Total soluble solids (TSS) content were determined by measuring the refractive index of fruit juice with a hand-held refractometer and the results expressed as percentages (Larrigaudiere et al., 2002).

2.5.3. Titratable acidity

Titrateable acidity (TA) content was measured by titrating 50 ml of the filtered liquid to pH 8.1 with 0.1 mol l⁻¹ NaOH and calculated as percent citric acid (Wright and Kader, 1997).

2.5.4. Ascorbic acid

Ascorbic acid (AA) content was determined using the 2,6-dichloro-indophenol method. Results were expressed as milligrams of ascorbic acid per 100 g fresh weight (Özden and Bayindirli, 2002).

2.5.5. Weight loss

Weight loss was determined before treatment (A) and after storage (B), and the weight loss was calculated as $(A - B)/A \times 100\%$.

2.5.6. External color

Strawberry external color was measured using a chromameter (CR-200; Minolta, Tokyo, Japan) and average readings of the L* (lightness), a* (– greenness to + redness), H (hue angle) values at four pre-determined points on the equatorial region of fruit were recorded.

2.6. Determination of population dynamics of the antagonists on the fruit surface

Sample fruit of each treatment were measured at harvest and after storage. Three fruits were randomly selected from each treatment group for each measurement cfu of *C. laurentii* and weight. Cloning forming units of *C. laurentii* were determined by dipping fruit in 100 ml of sterile water in a 250 ml Erlenmeyer flask, placing the flasks on a rotary shaker at 75 rpm for 10 min and 100 µL of serial 10-fold dilutions were spread on Rose Bengal agar plates and incubated at 28 °C for 2 days. The result was expressed as the Log₁₀ CFU per gram fresh weight.

2.7. Scanning electron microscopy (SEM) test of strawberry fruit epidermis

Three fruit were randomly removed from each treatment group, and two flakes (8 mm long and 8 mm wide) of strawberry fruit epidermis from the equatorial of each fruit with a razor blade, and then quickly immersed into 2.5% glutaraldehyde solution for 8 h at 4 °C. Prepared samples were observed by a scanning electron microscopy (HitachiS-3000N; Tokyo, Japan).

2.8. Statistical analyses

Data were expressed as mean ± standard deviation. SAS Software (Version 8.2; SAS Institute, Cary, NC, USA) was used for statistical analysis. One-way analysis of variance (ANOVA) was used to test statistical difference between treatments. Mean separations were performed by Duncan's multiple range tests. Statistical significance was assessed at the significance level $P = 0.05$.

3. Results

3.1. Efficacy of preharvest spraying *C. laurentii* on control of gray mold in strawberry fruit

Preharvest application of *C. laurentii* reduced gray mold disease incidence of strawberry fruit under ambient and refrigerated conditions (Fig. 2). After storage at 20 °C for 4 d, the diseases incidence in treatment C fruit was the lowest among the four treatment

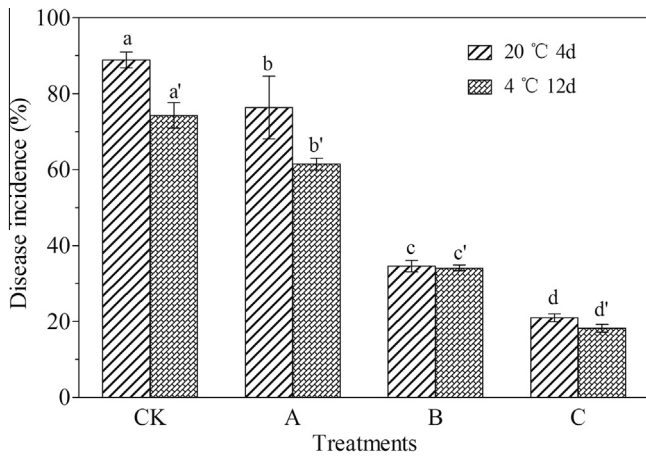


Fig. 2. Effect of preharvest *C. laurentii* treatment on gray mold decay of strawberry fruit caused by *Botrytis cinerea*. Data represent the means \pm SD. Different letters are significantly different according to Duncan's multiple range test at $P < 0.05$ level. CK: control; A: spraying of *C. laurentii* at 6d before harvest; B: spraying of *C. laurentii* at 6d and 0d before harvest; C: spraying of *C. laurentii* at 6, 3 and 0d before harvest.

groups. Also, disease incidence of treated fruit after storage at 4 °C for 12 days was significantly lower than those of the untreated control ($P < 0.05$). The best reduction gray mold incidence was achieved when fruit sprayed with *C. laurentii* three applications at 3-day intervals with first, 6 days before harvest and the last on the day of harvest.

3.2. Effect of preharvest spraying *C. laurentii* on postharvest natural decay of intact fruit

The effect of using *C. laurentii* before harvest on natural infection of postharvest fruit is shown in Fig. 3. Natural decay of all treated fruit was significantly lower than the control ($P < 0.05$) after storage 20 °C for 4 days or 4 °C for 12 days. In fruit treated with three applications of antagonist at 3-day intervals starting from 6 d before harvest, the percentage of decay fruit was the lowest in the four different treatment groups.

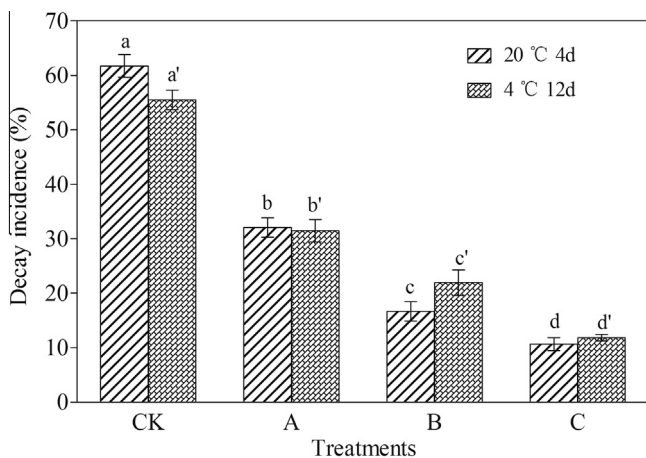


Fig. 3. Effect of preharvest spraying with *C. laurentii* on natural decay of strawberry fruit. Data represent the means \pm SD. Different letters are significantly different according to Duncan's multiple range test at $P < 0.05$ level. CK: control; A: spraying of *C. laurentii* at 6d before harvest; B: spraying of *C. laurentii* at 6d and 0d before harvest; C: spraying of *C. laurentii* at 6, 3 and 0d before harvest.

3.3. Effect of preharvest spraying *C. laurentii* on postharvest quality of strawberry fruit

As shown in Table 1, all fruit showed a decrease of L^* after storage. The L^* value of fruit sprayed with *C. laurentii* at 6, 3 and 0 d before harvest, was numerically higher than those of the control after storage 20 °C for 4 days or 4 °C for 12 days, but with not significantly difference ($P > 0.05$). No obvious difference in hue angle and a^* was found between treated fruit and the controls after storage. Our results showed that spraying with *C. laurentii* before harvest had no significant deleterious effects on the external color of strawberry.

Loss of weight in fresh fruit and vegetables is mainly due to the loss of water caused by respiration and transpiration processes. All fruit showed a progressive loss after storage (Table 2), but application with antagonist before harvest significantly reduced the weight loss. Using with antagonist at 6, 3 and 0 d before harvest significantly delayed the decrease of firmness (Table 2). In addition, preharvest spraying with *C. laurentii* also delayed the decrease of ascorbic acid, but had no significant effect on fruit total soluble solids and titratable acidity content on harvest day and after the storage at 20 °C for 4 days or 4 °C for 12 days.

3.4. Population dynamics of *C. laurentii* on strawberry fruit surface

The population dynamic of *C. laurentii* was investigated by determining the number of the yeast *C. laurentii* to colonize the fruit surface at harvest and after storage (Fig. 4). The frequency of spraying antagonist significantly influenced the population of yeast on the fruit surface. The number of yeast on strawberry fruit surface of treatment C was the highest in the four different treatment groups either at harvest or after storage.

3.5. Scanning electron microscopy images of strawberry fruit epidermis

The SEM images of strawberry fruit epidermis on harvest day is shown in Fig. 5. We observed some hyphae attached to the fruit epidermis in the control group (Fig. 5, CK), but no mycelium was observed on all treated fruit epidermis. This means that preharvest spraying with *C. laurentii* can reduce the pathogen infection when strawberry is grown in the greenhouse. The frequency of preharvest spraying *C. laurentii* influenced its colonization on the surface of strawberry fruit (Fig. 5). Treatment group sprayed with *C. laurentii* three times at 6, 3 and 0 d before harvest had the highest colonization density of the yeast.

4. Discussion

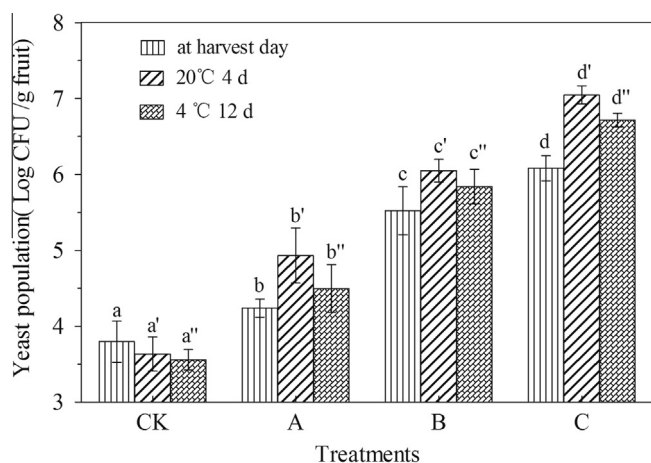
In this study, the efficacy of preharvest spraying with *C. laurentii* on postharvest decay of strawberry fruit was examined. The initial time for spraying was 6 days before harvest when strawberry fruit were at turning stage (Fig. 1) and susceptible to pathogenic micro-organism infections. Our results showed that the incidence of gray mold and natural decay in strawberry fruit after preharvest spraying with *C. laurentii* was significantly lower ($P < 0.05$) than that in the control after storage at 20 °C for 4 days and 4 °C for 12 days (Figs. 2 and 3). This finding was in accordance with previous studies, such as that of Zhao et al. (2008) and Tian et al. (2004). They reported that spraying with 10^8 CFU ml⁻¹ *Pichia guilliermondii* and *C. laurentii* efficiently reduced the postharvest decay of cherry tomato and sweet cherry fruit respectively, owing to the strong survival ability of antagonist on fruit surfaces under field conditions. Furthermore, our findings indicated that the biological control efficiency of preharvest spraying with *C. laurentii* was

Table 1Effect on external color of strawberry fruit with preharvest spraying of *C. laurentii*.

Storage condition	Treatments ^a	L ^{ab}	a [*]	H
Harvest day	CK	51.50 ± 1.63 ^{ab}	35.95 ± 1.36 ^{ab}	43.60 ± 1.49 ^{ab}
	A	53.04 ± 1.56 ^a	33.77 ± 1.37 ^b	46.80 ± 1.51 ^a
	B	50.98 ± 1.45 ^{ab}	35.38 ± 1.09 ^{ab}	44.50 ± 1.55 ^{ab}
	C	50.82 ± 1.49 ^{ab}	34.93 ± 1.18 ^{ab}	44.50 ± 1.43 ^{ab}
20 °C for 4 days	CK	49.03 ± 1.50 ^{ab}	37.06 ± 0.93 ^{ab}	41.58 ± 1.25 ^b
	A	47.98 ± 1.04 ^b	37.96 ± 0.61 ^a	42.01 ± 1.20 ^b
	B	47.08 ± 0.97 ^b	37.65 ± 0.59 ^a	41.63 ± 1.18 ^b
	C	48.69 ± 1.23 ^{ab}	36.54 ± 1.00 ^{ab}	42.99 ± 1.57 ^{ab}
4 °C for 12 days	CK	48.51 ± 1.44 ^a	37.62 ± 1.01 ^a	40.03 ± 1.51 ^a
	A	48.26 ± 1.24 ^a	38.80 ± 0.79 ^a	40.40 ± 1.17 ^a
	B	47.79 ± 1.05 ^a	38.48 ± 0.64 ^a	41.21 ± 1.15 ^a
	C	49.40 ± 1.08 ^a	38.86 ± 0.68 ^a	42.25 ± 1.05 ^a

^a CK: control; A: spraying of *C. laurentii* at 6 d before harvest; B: spraying of *C. laurentii* at 6 and 0 d before harvest; C: spraying of *C. laurentii* at 6, 3 and 0 d before harvest.^b Data represent the means ± SD, values followed by the different letter within column at the same storage condition are significantly different at $P = 0.05$ according to analysis by Duncan's multiple range test.**Table 2**Effect of preharvest spraying of *C. laurentii* on quality parameters of strawberry.

Storage condition	Treatments ^a	Weight loss ^b (%)	Firmness (N)	TSS (%)	TA (% citric acid)	AA (mg/100 g)
Harvest day	CK	0	2.70 ± 0.12 ^a	9.97 ± 0.19 ^a	0.81 ± 0.02 ^a	73.63 ± 1.21 ^a
	A	0	2.73 ± 0.10 ^a	10.15 ± 0.05 ^a	0.79 ± 0.03 ^a	73.45 ± 1.49 ^a
	B	0	2.88 ± 0.26 ^a	10.26 ± 0.06 ^a	0.83 ± 0.01 ^a	73.11 ± 1.75 ^a
	C	0	3.01 ± 0.27 ^a	10.18 ± 0.16 ^a	0.80 ± 0.02 ^a	74.58 ± 0.77 ^a
20 °C for 4 days	CK	12.12 ± 0.64 ^a	1.95 ± 0.19 ^b	8.11 ± 0.11 ^a	0.60 ± 0.01 ^a	41.72 ± 0.85 ^b
	A	10.19 ± 0.97 ^{ab}	2.43 ± 0.13 ^{ab}	8.29 ± 0.14 ^a	0.58 ± 0.04 ^a	42.55 ± 1.12 ^b
	B	10.91 ± 0.43 ^{ab}	2.39 ± 0.15 ^a	8.37 ± 0.15 ^a	0.63 ± 0.01 ^a	49.01 ± 0.96 ^a
	C	9.58 ± 0.47 ^b	2.62 ± 0.15 ^a	8.30 ± 0.14 ^a	0.61 ± 0.02 ^a	49.56 ± 1.00 ^a
4 °C for 12 days	CK	3.73 ± 0.16 ^a	2.35 ± 0.19 ^a	8.38 ± 0.10 ^a	0.59 ± 0.05 ^a	43.04 ± 1.38 ^a
	A	3.48 ± 0.22 ^a	2.42 ± 0.15 ^a	8.27 ± 0.13 ^a	0.57 ± 0.08 ^a	43.31 ± 1.85 ^a
	B	3.08 ± 0.28 ^b	2.54 ± 0.17 ^a	8.37 ± 0.30 ^a	0.61 ± 0.02 ^a	52.40 ± 1.67 ^b
	C	2.19 ± 0.12 ^b	2.84 ± 0.09 ^b	8.41 ± 0.22 ^a	0.63 ± 0.03 ^a	54.70 ± 0.87 ^b

^a CK: control; A: spraying of *C. laurentii* at 6 d before harvest; B: spraying of *C. laurentii* at 6 and 0 d before harvest; C: spraying of *C. laurentii* at 6, 3 and 0 d before harvest.^b Data represent the means ± SD, values followed by the different letter within column at the same storage condition are significantly different at $P = 0.05$ according to analysis by Duncan's multiple range test.**Fig. 4.** Population dynamics of *C. laurentii* on the surface of strawberry fruit. Data represent the means ± SD. Different letters are significantly different according to Duncan's multiple range test at $P < 0.05$ level. CK: control; A: spraying of *C. laurentii* at 6 d before harvest; B: spraying of *C. laurentii* at 6 and 0 d before harvest; C: spraying of *C. laurentii* at 6, 3 and 0 d before harvest.

positively correlated with its frequency of application. The result is consistent with a previous study (Larena et al., 2005), which demonstrated that the antagonist *Epicoccum nigrum* needed to be applied twice before harvest to reduce the incidence of postharvest brown rot in peaches. Therefore, given that different fruits have different growth cycles, we suggest that the frequency of

preharvest application of biocontrol agents should be increased appropriately.

For fruit quality, previous studies revealed that natural fungicides could decrease the decay index of fruit significantly with better quality attributes. For instance, Meng et al. (2010) observed that preharvest spray with *C. laurentii* combined with postharvest chitosan coating significantly decreased the weight loss of grape fruit. Preharvest using with Aloe Vera gel was effective in reducing the incidence of postharvest decay in table grapes, significantly reduced the fruit's respiration rate and weight loss, as well as delayed ripening parameters such as color and firmness (Castillo et al., 2010). Our results showed that spraying with *C. laurentii* three times at 6, 3 and 0 d before harvest could reduce the weight loss, delay the decrease of firmness and ascorbic acid, but had no significant effect on TSS, TA, as well as external color (Tables 1 and 2). It showed that preharvest spraying with *C. laurentii* could maintain the quality of strawberry fruit during storage.

Most pathogenic fungi causing postharvest diseases of fruit and vegetables attack wounds and cause decay immediately after harvest (Spotts et al., 1998). Therefore, application of antagonist as close as possible to the time of wounding should provide the best opportunity to protect the fruit (Roberts, 1994). Thus, timing of applications of antagonistic microorganisms and its ability to survive at sufficient populations on the fruit surface after application is vitally important (Benbow and Sugar, 1999). In the present study, our finding showed that the frequency of spraying with antagonist significantly influenced its ability to colonize on the surface of strawberry fruit (Figs. 4 and 5). Spraying with *C. laurentii* at 6, 3 and 0 d before harvest could improve the ability

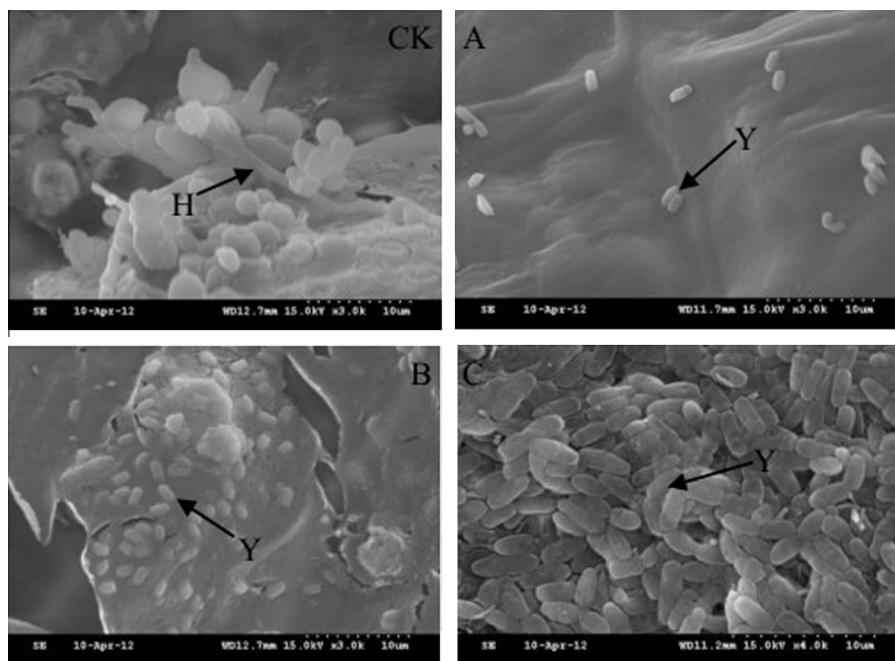


Fig. 5. SEM images of surface of strawberry fruits. CK: control; A: spraying of *C. laurentii* at 6 d before harvest; B: spraying of *C. laurentii* at 6 and 0 d before harvest; C: spraying of *C. laurentii* at 6, 3 and 0 d before harvest; H: hyphae; Y: yeast cells.

of antagonist to colonize epidermis of fruit. Preharvest application with antagonist is advantageous to colonize on the surface of fruit and compete with pathogen for nutrient and space so that it could reduce the invasion of pathogen in the greenhouse and during storage. This may be an important mechanism of preharvest application with antagonist to control postharvest diseases. The results of this work showed that preharvest application of antagonistic yeast would be an alternative to synthetic fungicides for controlling strawberry postharvest diseases.

In conclusion, preharvest spraying with *C. laurentii* was effective in reducing postharvest gray mold and natural decay of strawberry fruit. Three applications of *C. laurentii* at 6, 3 and 0 d before harvest could improve its ability to colonize epidermis of fruit, as well as the biological control efficiency. Moreover, preharvest spraying with *C. laurentii* could maintain fruit quality well. Future research will be aimed at the effect and mechanism of spraying with *C. laurentii* on fruit defense responses.

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